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(54) SYNERGISTICALLY STABILIZED LIQUID ENZYMATIC COMPOSITIONS
SYNERGISTISCH STABILISIERTE FLUESSIGE ENZYMATISCHE ZUSAMMENSETZUNGEN
COMPOSITIONS ENZYMATIQUES LIQUIDES STABILISEES DE MANIERE SYNERGIQUE

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Description

The present invention relates to novel formulations for stabilizing at least one enzyme contained in a liquid enzymatic composition. The unique rheological properties of the components of these stabilizing formulations preferably afford synergistic stabilizing capacity over other water-based mixtures of enzymatic dispersions, even under conditions of moderate to high heat and wide pH ranges. The invention, therefore, also relates to stabilized liquid enzymatic compositions. Additionally, the invention relates to novel methods for the preparation of stabilized liquid enzymatic compositions, and methods using the stabilizing formulations with liquid enzymatic compositions.

The use of enzymes and liquid enzymatic compositions in industry and in the commercial marketplace has grown rapidly over the last several years. As is well-known, enzymes can be acid, alkaline or neutral, depending upon the pH range in which they are active. All of these types of enzymes are contemplated to be useful in connection with the invention disclosed herein.

Many enzymes and liquid enzymatic compositions have been associated with liquid detergents and have shown utility as solubilizing and cleaning formulations. In addition to their association with liquid detergents, enzymes and liquid enzymatic compositions have also shown utility in a number of different commercial and industrial areas in which a wide variety of enzyme classes are now used.

Proteases are a well-known class of enzymes frequently utilized in a wide variety of industrial applications where they act to hydrolyze peptide bonds in proteins and proteinaceous substrates. Commercially, the greatest uses of proteases are made in the laundry detergent industry, where they help to remove protein-based stains such as blood or egg stains, and in the cheese-making industry, where they aid in curdling milk. Proteases are also used as meat tenderizers, for softening leather, for modifying food ingredients, and for flavor development. Liquid enzymatic compositions containing alkaline proteases have also shown to be useful as dispersants of bacterial films and algal and fungal mats in cooling tower waters and metalworking fluid containment bays.

Proteases can be characterized as acid, neutral, or alkaline proteases depending upon the pH range in which they are active. The acid proteases include the microbial rennets, rennin (chymosin), pepsin, and fungal acid proteases. The neutral proteases include trypsin, papain, bromelain/ ficin, and bacterial neutral protease. The alkaline proteases include subtilisin and related proteases. Commercial liquid enzymatic compositions containing proteases are available under the names Rennilase®, "PTN" (Pancreatic Trypsin NOVO), "PEM" (Proteolytic Enzyme Mixture), Neutrase®, Alcalase®, Esperase®, and Savinase™ which are all supplied by Novo Nordisk Bioindustrials, Inc. of Danbury, CT. Another commercial protease is available under the name HT-Proteolytic supplied by Solvay Enzyme Products.

Amylases, another class of enzymes, have also been utilized in many industrial and commercial processes in which they act to catalyze or accelerate the hydrolysis of starch. Amylases are used largely in the corn syrup industry for the production of glucose syrups, maltose syrups, and a variety of other more refined end products of starch hydrolysis such as high fructose syrups. As a class they include α-amylase, β-amylase, amyloglucosidase (glucoamylase), fungal amylase, and pullulanase. Commercial liquid enzymatic compositions containing amylases are available under the names BAN, Termamyl®, AMG, Fungamyl®, and Promozyme™, which are supplied by Novo Nordisk, and Diazyme L-200, a product of Solvay Enzyme Products.

Other commercially valuable enzyme classes are those which affect the hydrolysis of fiber. These classes include cellulases, hemicellulases, pectinases, and β-glucanases. Cellulases are enzymes that degrade cellulose, a linear glucose polymer occurring in the cell walls of plants. Hemicellulases are involved in the hydrolysis of hemicellulose which, like cellulose, is a polysaccharide found in plants. The pectinases are enzymes involved in the degradation of pectin, a carbohydrate whose main component is a sugar acid. β-glucanases are enzymes involved in the hydrolysis of β-glucans which are also similar to cellulose in that they are linear polymers of glucose. In a commercial context, these enzymes have utility to a greater or lesser degree in manufacturing processes dependent on fiber degradation.

Cellulases have reported utility in the de-inking process of old newsprint (ONP) wastepaper, eliminating the need for any surfactants and alkaline chemicals. The enzymes dislodge inks from fiber surfaces and disperse ink particles to a finite size. See S. Say-Kyoun Ow, *Biological De-Inking Methods of Newsprint Wastepaper,* World Pulp and Paper Technology, pp. 63, 64 (1992). Collectively, cellulases include endocellulase, exocellulase, exocello-biohydrolase, mannase, and cellobiase. Commercial liquid enzymatic compositions containing cellulases are available under the names Celluclast® and Novozym®188 which are both supplied by Novo Nordisk.

Hemicellulases are also used in the de-inking process to dislodge ink particles from the fiber surface of ONP. See D. Y. Prasad et al., *Enzyme Deinking of Black and White Letterpress Printed Newsprint Waste,* Progress in Paper Recycling, May 1992, pp. 21, 22. Additionally, hemicellulases, such as the xylanases, are employed in the pulp bleaching process. Xylanase pretreatment of kraft pulps has resulted in major reductions in bleaching chemical requirements, such as molecular chlorine, and has also improved pulp quality as reflected by higher brightness ceilings. See D. J. Senior et al., *Reduction in Chlorine Use During Bleaching of Kraft Pulp Following Xylanase Treatment,* Tappi Journal (forthcoming publication; aspects of the publication were presented at the 1991 International Pulp Bleaching Conference, Stockholm). PULPZYM® product, available from Novo Nordisk, and ECOPULP® product, from Alko Biotechnol-

ogy, are two examples of commercially available liquid enzymatic compositions containing xylanase-based bleaching enzymes.

As a class, hemicellulases include hemicellulase mixture and galactomannanase. Commercial liquid enzymatic compositions containing hemicellulases are available as PULPZYM® from Novo, ECOPULP® from Alko Biotechnology and Novozym®280 and Gamanase™, which are both products of Novo Nordisk.

The pectinases are used commercially to weaken cell walls and enhance extraction of fruit juice, as well as to aid in decreasing viscosity and preventing gelation in these extracts. Pectinases consist of endopolygalacturonase, exopoly-galacturonase, endopectate lyase (transeliminase), exopectate lyase (transeliminase), and endopectin lyase (transeliminase). Commercial liquid enzymatic compositions containing pectinases are available under the names Pectinex™ Ultra SP and Pectinex™*, both supplied by Novo Nordisk.

The β-glucanases play an important role in the malting and brewing industries where modification of barley cell walls containing β-glucans is necessary. β-glucanases are comprised of lichenase, laminarinase, and exoglucanase. Commercial liquid enzymatic compositions containing β-glucanases are available under the names Novozym®234, Cereflo®, BAN, Finizym®, and Ceremix®, all of which are supplied by Novo Nordisk.

Two additional classes of industrially and commercially useful enzymes are lipases and phospholipases. Lipases and phospholipases are esterase enzymes which hydrolyze fats and oils by attacking the ester bonds in these compounds. Lipases act on triglycerides, while phospholipases act on phospholipids. In the industrial sector, lipases and phospholipases represent the commercially available esterases, and both currently have a number of industrial and commercial applications.

In the pulp and paper industry, liquid enzyme preparations containing lipases have proven to be particularly useful in reducing pitch deposits on rolls and other equipment during the production process. For example, the treatment of unbleached sulfite pulp with lipases prior to bleaching with chlorine to reduce the content of chlorinated triglycerides, which are reportedly the cause of pitch deposition during the paper manufacturing process, has been reported. See K. Fischer and K. Messner, *Reducing Troublesome Pitch in Pulp Mills By Lipolytic Enzymes,* Tappi Journal, Feb. 1992, p. 130. Novo Nordisk markets two liquid lipase preparations under the names Resinase™ A and Resinase™ A 2X, both of which, under certain conditions, reportedly reduce pitch deposits significantly by breaking down wood resins in pulp.

Another important use of lipases is to degrease hides and pelts in the leather-making process. Alkaline lipases are used in conjunction with special proteases and emulsifying systems to aid degreasing, as well as to improve the soaking and liming effect in leather-making. See J. Christner, *The Use of Lipases in the Beamhouse Processes,* 87 J. A.L.C.A. 128 (1992).

Lipases have also been used for the development of flavors in cheese and to improve the palatability of beef tallow to dogs. In nonaqueous systems, lipases have been employed to synthesize esters from carboxylic acids and alcohols.

Commercial liquid enzymatic compositions containing lipases are available. For example, such compositions are available under the trade names Lipolase 100, Greasex 50L, Palatase™A, Palatase™M, and Lipozyme™ which are all supplied by Novo Nordisk.

With respect to the commercially useful phospholipases, pancreatic phospholipase A_2 has been used to convert lecithin into lysolecithin. Lysolecithin reportedly is an excellent emulsifier in the production of mayonnaise and the baking of bread. Commercially, phospholipase A_2 is available in a liquid enzymatic composition sold as LECITASE™ by Novo Nordisk.

Another commercially valuable class of enzymes are the isomerases which catalyze conversion reactions between isomers of organic compounds. The isomerases are particularly important in the high fructose corn syrup industry. For example, the aldose-ketose isomerase reaction, catalyzed by glucose isomerase, involves the conversion of glucose to fructose and is just one of three key enzyme reactions in the industry. Sweetzyme® product is a liquid enzymatic composition containing glucose isomerase which is supplied by Novo Nordisk.

Redox enzymes are enzymes that act as catalysts in chemical oxidation/reduction reactions and, consequently, are involved in the breakdown and synthesis of many biochemicals. Currently, many redox enzymes have not gained a prominent place in industry since most redox enzymes require the presence of a cofactor. However, where cofactors are an integral part of an enzyme or do not have to be supplied, redox enzymes are commercially useful, particularly in the food processing industry.

The redox enzyme, glucose oxidase, is used to prevent unwanted browning reactions affecting food color and flavor. Glucose oxidase is also used as an "oxygen scavenger" to prevent the development of off-flavors in juices and to preserve color and stability in certain sensitive food ingredients. The redox enzyme, catalase, has been utilized to decompose residual hydrogen peroxide used as a sterilizing agent. A third redox enzyme, lipoxidase (lipoxygenase), found naturally in soya flour and not usually purified for industrial use, is used in baking not only to obtain whiter bread, but also to reverse the dough softening effects caused by certain agents. Other redox enzymes have possible applications ranging from the enzymatic synthesis of steroid derivatives to use in diagnostic tests. These redox enzymes include peroxidase, superoxide dismutase, alcohol oxidase, polyphenol oxidase, xanthine oxidase, sulfhydryl oxidase,

hydroxylases, cholesterol oxidase, laccase, alcohol dehydrogenase, and steroid dehydrogenases.

When enzymes, such as those described above, are prepared or sold for use in industrial processes, they generally are formulated as water-based or aqueous liquid enzymatic compositions designed for a particular process. Water-based liquid enzymatic compositions may contain additional solvents depending upon the particular enzyme or use of the composition. These liquid enzymatic compositions, however, have historically been plagued with problems such as chemical instability which can result in the loss of enzymatic activity, particularly upon storage. This critical problem of loss of enzymatic activity upon storage has particularly affected the liquid detergent industry.

It is not uncommon to have industrial products, such as liquid enzymatic compositions, stored in warehouses in various climates around the world where the product is subjected to a temperature that may range from freezing to above 50°C for extended periods. After storage at temperature extremes ranging from 0°C to 50°C for many months, most liquid enzymatic compositions lose from 20 to 100 percent of their enzymatic activity due to enzyme instability.

Various attempts have been made to stabilize enzymes contained in liquid enzymatic compositions. Attempts to increase the stability of liquid enzymatic compositions using formulations containing alcohols, glycerols, dialkylglycolethers, and mixtures of these and other compounds have had only marginal success, even in moderate storage temperature ranges.

In U.S. Patent No. 4,801,544, a system of ethylene glycol and ethoxylated linear alcohol nonionic surfactant with hydrocarbon solvent was utilized as a stabilizer, and the encapsulation of enzymes in micelles within the solvent/surfactant mixture was described. The water content of the composition was kept at less than 5 percent, and enzyme stability was checked at 35°, 70° and 100°F.

The stabilization of an aqueous enzyme preparation using certain esters has been described in U.S. Patent No. 4,548,727. The ester used as a stabilizer has the formula, RCOOR', where R is an alkyl of from one to three carbons or hydrogen, and R' is an alkyl of from one to six carbons. The ester is present in the aqueous enzyme preparation in an amount from 0.1 to about 2.5% by weight.

U.S. Patent No. 4,318,818 describes a stabilizing system for aqueous enzyme compositions where the stabilizing system comprises calcium ions and a low molecular weight carboxylic acid or its salt. The pH of the stabilizing system is from about 6.5 to about 10.

U.S. Patent No. 4,243,543 teaches the stabilization of liquid proteolytic enzyme-containing detergent compositions. The detergent compositions are stabilized by adding an antioxidant and a hydrophilic polyol to the composition while stabilizing the pH of the composition.

U.S. Patent No. 4,169,817 teaches a liquid cleaning composition containing stabilized enzymes. The composition is an aqueous solution containing from 10% to 50% by weight of solids and including detergent builders, surface active agents, an enzyme system derived from *Bacillus subtilis* and an enzyme stabilizing agent. The stabilizing agents comprise highly water soluble sodium or potassium salts and/or water soluble hydroxy alcohols and enable the solution to be stored for extended periods without deactivation of the enzymes.

European Patent No. 0 352 244 A2 describes stabilized liquid detergent compositions using an amphoteric surfactant.

The present invention provides a formulation capable of synergistically stabilizing one or more enzymes contained in a liquid enzymatic composition.

The invention, thus, also provides stabilized liquid enzymatic compositions.

Additionally, the invention provides methods for the preparation of stabilized liquid enzymatic compositions.

The various aspects of the invention can be broadly accomplished by the use of a formulation for stabilizing a liquid enzymatic composition comprising:

(a) at least one poly(cellulosic)ether,
(b) a C_2-C_6 polyhydric alcohol, and
(c) water,

wherein components (a) and (b) are present in an amount effective to stabilize at least one enzyme contained in a liquid enzymatic composition. More preferably, the polymer (a) and the C_2-C_6 polyhydric alcohol (b) are present in a combined amount synergistically effective to stabilize at least one enzyme contained in a liquid enzymatic composition.

The inventive stabilizing formulation can be used with a wide variety of enzymes utilized in liquid enzymatic compositions performing a wide variety of functions. The enzymes and classes of enzymes with which this stabilizing formulation can be used include, but are not limited to, those discussed above.

The invention also relates to a stabilized liquid enzymatic composition comprising:

(a) at least one poly(cellulosic)ether,
(b) a C_2-C_6 polyhydric alcohol,

(c) water, and
(d) at least one enzyme;

wherein components (a) and (b) are present in an amount effective to stabilize at least one enzyme in the liquid enzymatic composition. More preferably, the polymer (a) and the C_2-C_6 polyhydric alcohol (b) are present in a combined amount synergistically effective to stabilize at least one enzyme contained in the liquid enzymatic composition.

The invention further relates to a method for the preparation of a stabilized liquid enzymatic composition by combining an enzyme with the stabilizing formulation above. Additionally, the invention relates to a method of using the stabilizing formulation to stabilize a liquid enzymatic formulation comprising the step of combining the stabilizing formulation with a liquid enzymatic composition.

In a preferred embodiment, the invention provides a formulation for stabilizing a liquid enzymatic composition comprising:

(a) at least one poly(cellulosic)ether,
(b) a C_2-C_6 polyhydric alcohol, and
(c) water,

wherein components (a) and (b) are present in a combined amount synergistically effective to stabilize at least one enzyme contained in a liquid enzymatic composition.

A water-soluble, or at least partially water-soluble, polymer is used in a formulation for stabilizing a liquid enzymatic composition or a stabilized liquid enzymatic compositions of the invention. That is, the polymer must have sufficient solubility to be miscible with water and form a single phase. Having this solubility, the polymer should not separate out when combined with the C_2-C_6 polyhydric alcohol and water of a stabilizing formulation or with a liquid enzymatic composition. The formulation is not required to be a clear solution. Preferably, the formulation is an emulsion having an apparent homogeneous texture.

In a formulation for stabilizing a liquid enzymatic composition or stabilized liquid enzymatic composition of the invention, the amount of polymer present also depends on the molecular weight of the particular polymer used. The higher the molecular weight of the polymer used, the lower the amount of polymer generally required to stabilize an enzyme.

The polymer may preferably be used in amounts up to about 50% by weight of the stabilizing formulation. More preferably, the polymer is present from 0.05 to 30% by weight, and most preferably from 1% to 10% by weight. In a preferred embodiment, the polymer, of course, is present in an amount that gives the desired synergistic stabilization of a liquid enzymatic composition when combined with the polyhydric alcohol in a water-based or aqueous formulation.

The polymer employed in the present invention is poly(cellulosic)ethers. The polymer may be substituted or unsubstituted. The polymer may have a slight ionic charge, but is preferably non-ionic in nature.

The polymer is preferably selected from poly(carboxymethylcellulose)ether, poly(hydroxypropylmethylcellulose) ether, poly(hydroxyethylmethylcellulose)ether, poly(hydroxybutylmethylcellulose)ether, poly(hydroxypropylcellulose) ether, and poly(ethylhydroxyethylcellulose)ether. More preferably, the polymer is poly(carboxymethylcellulose)ether. Certain poly(cellulosic)ethers used in the present invention, such as poly(carboxymethylcellulose)ether, are sold as salts, i.e. sodium salts, and possess a slight anionic charge. Preferable poly(cellulosic)ethers are those with molecular weights ranging from 15,000 to 100,000, but more preferred are those with molecular weights ranging from 20,000 to 75,000.

The stabilizing formulation also contains a C_2-C_6 polyhydric alcohol as a second component. The C_2-C_6 polyhydric alcohol acts synergistically with the polymer, described above, to stabilize an enzyme in a liquid enzyme composition. The C_2-C_6 polyhydric alcohol is preferably selected from a glycol and a trihydric alcohol. More preferably, the C_2-C_6 polyhydric alcohol is glycerol, sorbitol, propylene glycol, butylene glycol, hexylene glycol, or ethylene glycol. Most preferably, the C_2-C_6 polyhydric alcohol is glycerol.

The stabilizing formulation contains the C_2-C_6 polyhydric alcohol in an amount sufficient, with the polymer, to stabilize at least one enzyme in a liquid enzymatic composition. Preferably, the C_2-C_6 polyhydric alcohol present is 0.50 to 60% by weight of the stabilizing formulation, more preferably, 5 to 50% by weight and, even more preferably, between 10 and 30%. Most preferably, a stabilizing formulation or enzymatic composition contains the C_2-C_6 polyhydric alcohol in a combined amount with the polymer to achieve synergistic stabilization.

The formulations of the invention are water-based or aqueous formulations containing sufficient water to allow the polymer to be miscible with the formulation and not separate out. Generally, the C_2-C_6 polyhydric alcohol is soluble in water, but sufficient water should be present to allow the formulation to form a single phase. As discussed above, the formulation is not required to be a clear solution and preferably is an emulsion having an apparent homogeneous texture. Water-based formulations may contain additional solvents other than water.

While the stabilizing formulations of the invention can be prepared by mixing the components in any order, the

formulations are preferably prepared by adding the desired amount of polymer to a C_2-C_6 polyhydric alcohol/water mixture. The C_2-C_6 polyhydric alcohol/water mixture can be prepared by means known in the art. For example, the mixture can be prepared by simply mixing the desired polyhydric alcohol with an appropriate amount of water, or diluting a previously prepared mixture.

The preferred mixture of the C_2-C_6 polyhydric alcohol and water may contain any percentage of C_2-C_6 alcohol sufficient with the polymer discussed above to stabilize, preferably synergistically, at least one enzyme in a liquid enzymatic composition. Preferably, the mixture is 1-95% by weight of the polyhydric alcohol, or water-soluble polymer thereof; more preferably 10-50% by weight; and most preferably, 30-50% by weight. When the polyhydric alcohol is glycerol, the mixture is preferably a 50% by weight glycerol/ water mixture. Not being bound to a particular theory, applicant believes that the mixture acts to wet the polymer used in the present invention and the C_2-C_6 polyhydric alcohol in the mixture, to synergistically stabilize an enzyme.

To adequately stabilize an enzyme in a liquid enzymatic composition according to the invention, the enzyme should possess at least 90% activity after 30 days at 25°C. The examples below demonstrate the preferred synergistic stabilization of various enzymes at 50°C after 30 days.

The stabilizing formulation described here can be employed with a wide variety of enzymes and industrial processes or commercial products. The enzymes, industrial processes and commercial products with which this stabilizing formulation can be used include, but are not limited to, those previously discussed.

The use of the stabilizing formulation to stabilize a liquid enzymatic composition results in a second embodiment of this invention, a stabilized liquid enzymatic composition. Thus, the invention also relates to a stabilized liquid enzymatic composition comprising at least one poly(cellulosic)ether; a C_2-C_6 polyhydric alcohol; water; and at least one enzyme. The polymer and C_2-C_6 polyhydric alcohol are present in a combined amount effective to stabilize, preferably to synergistically stabilize, at least one enzyme contained in the liquid enzymatic composition. Preferred compositions according to the invention are capable of developing greater viscosities than quantitatively proportional aqueous mixtures with the same polymers.

The contemplated and preferred embodiments regarding the polymer, the C_2-C_6 polyhydric alcohol, and water present in this stabilized liquid enzymatic composition are the same as those discussed above with respect to the stabilizing formulation of this invention.

As with the stabilizing formulation, the liquid enzymatic composition of this invention can be practiced with a wide variety of enzymes. These enzymes include, but are not limited to, the enzyme classes and specific enzymes heretofore discussed. Enzymes that may be used are derived from animal, plant, fungal, bacterial, and synthetic sources. Preferable water dispersible enzymes for this system are proteases, including acid, alkaline, and neutral proteases, which are widely used in the laundry detergent and cheese making industries; amylases, including acid, alkaline, and neutral amylases, used, for example, in the corn syrup industry; lipases, used in developing flavors in cheese, and in the pulp and paper and leather making industries; cellulases, and xylases.

Depending on the intended use, enzymes are often packaged and sold in concentrated liquid enzymatic compositions to be diluted prior to use. Enzymes can also be supplied in powdered or desiccated form. The amount of enzyme present after dilution of the concentrated enzyme depends on the form in which the enzyme is supplied. In general, the amount of enzyme preferably may range from 0.05 to 40% by weight of a concentrated liquid enzymatic composition, more preferably 0.5 to 25%, and most preferably 10 to 20%. The stabilizing formulation of this invention is specifically contemplated for use in concentrated liquid enzymatic compositions as well as with compositions already diluted for use. The amount of stabilizing formulation needed to stabilize, or to synergistically stabilize, a concentrated solution can, and most likely will, differ from that for a diluted composition. Determining the appropriate quantity of stabilizing formulation or its components can be readily ascertained by one of ordinary skill in the art using the method set out in the examples below. As known in the art, the amount of enzyme present, however, is dependent upon the activity of the particular enzyme and the desired end use.

Depending upon the enzyme it contains and its intended use, the pH of the final stabilized liquid enzymatic composition is preferably from 5.0 to 10.0, but more preferably around 7.0. Most preferably, the system should be allowed to seek its own pH, generally around neutral. But, as understood in the art, adjustment of pH may be necessary with a small amount of acidic or alkaline material.

The stabilized liquid enzymatic composition may be water-based or aqueous and contain other solvents or additives directed toward the use of the composition in a particular industrial process. For example, the stabilized liquid enzymatic composition can contain additives such as surfactants, defoamers, and the like, as are known in the art. With synergistic stabilization, such additives may be added in amounts not interfering with the synergistic stabilization of the liquid enzymatic composition. One skilled in the art can readily determine such amounts. Advantageously, when a stabilized liquid enzymatic composition of the invention is used, the stabilizing formulation may also act as a dispersant aid for the enzyme in industrial process waters.

The invention also relates to a method for the preparation of a stabilized liquid enzymatic composition comprising the step of combining at least one enzyme with the inventive stabilizing formulation. The invention further relates to a

method of using the stabilizing formulation to stabilize a liquid enzymatic composition comprising the step of combining a liquid enzymatic composition with the stabilizing formulation. Illustrative and preferred components, as well as the amounts of the components used in the method, are the same as discussed above.

One of ordinary skill would understand that the components of the formulation for stabilizing a liquid enzymatic composition or the stabilized liquid enzymatic composition can be combined in any order or even simultaneously. However, the following order is preferred:

(a) mixing a C_2-C_6 polyhydric alcohol with water,
(b) adding to the mixture prepared in step (a) at least one poly(cellulosic)ether and
(c) adding at least one enzyme to the mixture resulting from step (b). The polymer and C_2-C_6 polyhydric alcohol are preferably present in a combined amount synergistically effective to stabilize at least one enzyme in the resulting liquid enzymatic composition.

An alternative method for the preparation of a stabilized liquid enzymatic composition comprises the steps of:

(a) mixing a C_2-C_6 polyhydric alcohol with water,
(b) adding to the mixture prepared in step (a) at least one poly(cellulosic)ether and
(c) combining the formulation resulting from steps (a) and (b) with a liquid enzymatic composition containing at least one enzyme. The polymer and C_2-C_6 polyhydric alcohol are preferably present in a combined amount synergistically effective to stabilize at least one enzyme in the liquid enzymatic composition.

In the methods according to the invention, the polymer added in step (b) may be added alone, as an aqueous dispersion, or as a solution where the polymer is dissolved in water or a suitable organic solvent. When other additives are to be included in the stabilized liquid enzymatic composition, such additives may be added at any time, but preferably prior to step (c), or in a separate step after the enzyme is added.

The following examples are given to illustrate the invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details set forth in these examples.

EXAMPLE 1

Synergism was demonstrated by testing glycerol, designated as Compound A, and poly(carboxymethylcellulose) ether, (CMC), having a 250,000 molecular weight, designated as Compound B. As illustrated by Tables 1 and 2, experiments were set up by varying ratios of Compound A to Compound B over a range of concentrations and assaying various types of enzymes for enhanced stabilization of enzymatic activity at 50°C for 30 days. The concentration of each compound required for an assay of 90% of the original enzymatic activity was taken as an end point. The concentrations are expressed as percent by weight of the compound in the final composition including the added enzyme with the balance being water. Water was added to compound A, the glycerol to form a glycerol-water mixture. End points for the compositions containing Compound A and Compound B were then compared with the end points for Compound A alone and Compound B alone.

Synergism was determined by the method described by Kull, F.C., Eisman, P.C., Sylwestrowicz, H.D., and Mayer, R.L., Applied Microbiology 9: 538-541 (1961) employing the ratio determined by

$$\frac{QA}{Qa} + \frac{QB}{Qb}$$

where

Qa = Percentage (by weight) of aqueous mixture of Compound A, acting alone, which produced an end point,
Qb = Percentage (by weight) of aqueous mixture of Compound B, acting alone, which produced an end point,
QA = Percentage (by weight) of aqueous mixture of Compound A to Compound B, which produced an end point, and
QB = Percentage (by weight) of aqueous mixture of Compound B to Compound A, which produced an end point.

Where the sum of QA/Qa and QB/Qb is greater than one, antagonism is indicated. If the sum is equal to one, additivity is indicated. Where the sum is less than one, synergism is demonstrated.

This procedure for demonstrating the synergism of the compositions of this invention is a widely used and accepted procedure. More detailed information is provided in the article by Kull et al. Further information concerning this procedure is contained in U.S. Pat. No. 3,231,509, the disclosure of which is incorporated here by reference.

The results obtained, which are set forth in Tables 1 and 2, demonstrate the enhanced stabilization of the enzyme,

HT-Proteolytic L-175, an alkaline protease sold by Solvay Enzymes, Inc. Using the method described by Kull et al., the sums of QA/Qa + QB/Qb for all compositions containing glycerol (Compound A) and CMC (Compound B) were calculated. Sample calculations are shown for some end points where synergism was evident in this example. As set forth in Table 1 and the sample calculations, these end point values were 0.58, 0.67, 0.67 and 0.67, respectively, indicating the existence of synergism.

TABLE 1

Compound A (% by weight)						
	60	30	20	10	5	0
Compound B (% by weight)						
6	+	+	+	+	+	+*
3	+	+	+	+*	+*	-
2	+	+	+*	-	-	-
1	+	+*	-	-	-	-
0	+*	-	-	-	-	-

(1)(*) = End point of ≥ 90% enzymatic activity after 30 days at 50°C
(2)(+) = ≥ 90% enzymatic activity after 30 days at 50° C
(3)(-) = < 90% enzymatic activity remaining after 30 days at 50°C
(4) Compound A = Glycerol
(5) Compound B = CMC

Calculations:				
Qa	Qb	QA	QB	QA/Qa + QB/Qb
(%) 60	6	5	3	5/60 + 3/6 = 0.58
		10	3	10/60 + 3/6 = 0.67
		20	2	20/60 + 2/6 = 0.67
		30	1	30/60 + 1/6 = 0.67

EXAMPLE 2

Stabilizing formulations of the invention as in Example 1 were tested with another enzyme, Diazyme L-200, a glucoamylase sold by Solvay Enzymes, Inc. The results, set forth in Tables 3 and 4, also demonstrate enhanced stabilization with this enzyme. The sums of QA/Qa + QB/Qb for all compositions containing glycerol (Compound A) and CMC (Compound B) were calculated. Sample calculations are shown for some end points where synergism was evident in this example. As set forth in Table 2 and the sample calculations, these end point values were 0.59, 0.67, 0.67, and 0.84, respectively, indicating the existence of synergism.

TABLE 2

	Compound A (% by weight)					
	60	30	20	10	5	0
Compound B (% by weight)						
6	+	+	+	+	+	+*
3	+	+	+	+*	+*	-
2	+	+*	+*	-	-	-

TABLE 2 (continued)

	Compound A (% by weight)					
	60	30	20	10	5	0
Compound B (% by weight)						
1	+	-	-	-	-	-
0	+*	-	-	-	-	-

(1)(*) = End point of ≥ 90% enzymatic activity after 30 days at 50°C
(2)(+) = ≥ 90% enzymatic activity after 30 days at 50°C
(3)(-) = < 90% enzymatic activity remaining after 30 days at 50°C
(4) Compound A = Glycerol
(5) Compound B = CMC

Calculations:

Qa	Qb	QA	QB	QA/Qa + QB/Qb
(%) 60	6	5	3	5/60 + 3/6 = 0.59
		10	3	10/60 + 3/6 = 0.67
		20	2	20/60 + 2/6 = 0.67
		30	2	30/60 + 2/6 = 0.84

EXAMPLE 3

Enzymes with esterase activity were tested with stabilizing formulations according to the invention as in Example 1. The results obtained with the enzyme, Lipolase, a lipase sold by Novo Nordisk Bioindustries, Inc., are set forth in Table 3 and indicate the enhanced stabilization of this enzyme. The sums of QA/Qa + QB/Qb for all compositions containing aqueous glycerol (Compound A) and CMC (Compound B) were calculated. Sample calculations are shown for some end points where synergism was evident in this example. As set forth in Table 3 and the sample calculations, these end point values were 0.43, 0.37, 0.57, and 0.77, respectively, indicating the existence of synergism.

TABLE 3

	Compound A (% by weight)					
	50	30	20	10	5	0
Compound B (% by weight)						
6	+	+	+	+	+	+*
3	+	+	+	+	+	-
2	+	+	+	+	+*	-
1	+	+*	+*	+*	-	-
0	+*	-	-	-	-	-

(1)(*) = End point of ≥ 90% enzymatic activity after 30 days at 50°C
(2)(+) = ≥ 90% enzymatic activity after 30 days at 50°C
(3)(-) = < 90% enzymatic activity remaining after 30 days at 50°C
(4) Compound A = Glycerol
(5) Compound B = CMC

Calculations:				
Qa	Qb	QA	QB	QA/Qa + QB/Qb
(%) 50	6	5	2	5/50 + 2/6 = 0.43
		10	1	10/50 + 1/6 = 0.37
		20	1	20/50 + 1/6 = 0.56
		30	1	30/50 + 1/6 = 0.76

EXAMPLE 4

The hemicellulase, Pulpzyme HB, a xylanase sold by Novo Nordisk Bioindustrials, Inc., was selected for enhanced stability formulation testing as in Example 1. The following results obtained for this example are set forth in Table 4. The sums of QA/Qa + QB/Qb for all compositions containing glycerol (Compound A) and CMC (Compound B) were calculated. Sample calculations are shown for those end points where synergism was evident in this example. As set forth in Table 7 and the sample calculations, these end point values were 0.43, 0.53, 0.57, and 0.77, respectively, indicating the existence of synergism.

TABLE 4

	Compound A (% by weight)					
	50	30	20	10	5	0
Compound B (% by weight)						
6	+	+	+	+	+	+*
3	+	+	+	+	+	-
2	+	+	+	+*	+*	-
1	+	+*	+*	-	-	-
0	+*	-	-	-	-	-
(1)(*) = End point of ≥ 90% enzymatic activity after 30 days at 50°C (2)(+) = ≥ 90% enzymatic activity after 30 days at 50°C (3)(-) = < 90% enzymatic activity remaining after 30 days at 50°C (4) Compound A = Glycerol (5) Compound B = CMC						

Calculations:				
Qa	Qb	QA	QB	QA/Qa + QB/Qb
(%) 50	6	5	2	5/50 + 2/6 = 0.43
		10	2	10/50 + 2/6 = 0.53
		20	1	20/50 + 1/6 = 0.57
		30	1	30/50 + 1/6 = 0.77

Claims

1. A formulation for stabilizing a liquid enzymatic composition comprising:

   (a) at least one poly(cellulosic)ether,
   (b) a C_2-C_6 polyhydric alcohol, and
   (c) water,

wherein components (a) and (b) are present in an amount effective to stabilize at least one enzyme contained in a liquid enzymatic composition.

2. The formulation of claim 1 wherein said poly(cellulosic)ether is selected from poly(carboxymethylcellulose)ether, poly(hydroxypropylmethylcellulose)ether, poly(hydroxyethylmethylcellulose)ether, poly(hydroxybutylmethylcellulose)ether, poly(hydroxypropylcellulose)ether, and poly(ethylhydroxyethylcellulose)ether.

3. The formulation of claim 1 wherein said C_2-C_6 polyhydric alcohol is glycerol, sorbitol, propylene glycol, butylene glycol, hexylene glycol, or ethylene glycol and said components (b) and (c) are contained in a mixture of 1-95% by weight of said C_2-C_6 polyhydric alcohol and the remainder water.

4. The formulation of any one of claims 1 to 3 wherein components (a) and (b) are present in a combined amount synergistically effective to stabilize at least one enzyme contained in a liquid enzymatic composition.

5. A stabilized liquid enzymatic composition comprising a formulation according to any one of claims 1 to 4 and at least one enzyme.

6. The composition of claim 5 wherein said composition is an emulsion.

7. A stabilized liquid enzymatic composition comprising a formulation as claimed in any one of claims 1 to 4 and at least one enzyme.

8. A method for preparing a liquid stabilized enzymatic composition comprising the step of combining at least one enzyme and a formulation as claimed in any one of claims 1 to 4.

9. The method of claim 8 further comprising, before said combining step, the steps of mixing said C_2-C_6 polyhydric alcohol and said water, and
adding said poly(cellulosic)ether to said C_2-C_6 polyhydric alcohol and water mixture.

10. The method of claim 8 or 9 wherein said C_2-C_6 polyhydric alcohol is glycerol, sorbitol, propylene glycol, butylene glycol, hexylene glycol, or ethylene glycol; said mixture is 1-95% by weight of said C_2-C_6 polyhydric alcohol and the remainder water; and said at least one enzyme is selected from a protease, amylase, lipase, cellulase, mannase, and xylase.

11. Use of a formulation according to any one of claims 1 to 4, to stabilize a liquid enzymatic composition comprising the step of combining a liquid enzymatic composition containing at least one enzyme with said formulation.

12. Use according to claim 13 wherein said poly(cellulosic)ether is selected from poly(carboxymethylcellulose)ether and said C_2-C_6 polyhydric alcohol is glycerol and said at least one enzyme is selected from a protease, amylase, lipase, cellulase, mannase, and xylase.

Patentansprüche

1. Eine Formulierung zum Stabilisieren einer flüssigen enzymatischen Zusammensetzung, umfassend:

(a) mindestens einen Poly(cellulose)ether
(b) einen C_2-C_6 mehrwertigen Alkohol und
(c) Wasser,

wobei Komponenten (a) und (b) in einer Menge anwesend sind, die wirksam ist, um mindestens ein Enzym zu stabilisieren, enthalten in einer flüssigen enzymatischen Zusammensetzung.

2. Formulierung nach Anspruch 1, wobei der Poly(cellulose)ether ausgewählt ist aus

Poly(carboxymethylcellulose)ether,
Poly(hydroxypropylmethylcellulose)ether,
Poly(hydroxyethylmethylcellulose)ether,

Poly(hydroxybutylmethylcellulose)ether,
Poly(hydroxypropylcellulose)ether und
Poly(ethylhydroxyethylcellulose)ether.

3. Formulierung nach Anspruch 1, wobei der C_2-C_6 mehrwertige Alkohol Glycerol, Sorbitol, Propylenglykol, Butylenglykol, Hexylenglykol oder Ethylenglykol ist und die Komponenten (b) und (c) in einer Mischung von 1-95 Gew.-% des C_2-C_6 mehrwertigen Alkohols und des Restwassers enthalten sind.

4. Formulierung nach mindestens einem der Ansprüche 1 bis 3, wobei Komponenten (a) und (b) in einer kombinierten Menge vorhanden sind, die synergistisch wirksam ist, um mindestens ein Enyzm zu stabilisieren, enthaltend in einer flüssigen enzymatischen Zusammensetzung.

5. Stabilisierte flüssige enzymatische Zusammensetzung, umfassend eine Formulierung gemäß mindestens einem der Ansprüche 1 bis 4 und mindestens ein Enzym.

6. Zusammensetzung nach Anspruch 5, wobei die Zusammensetzung eine Emulsion ist.

7. Stabilisierte flüssige enzymatische Zusammensetzung, umfassend eine Formulierung, wie in mindestens einem der Ansprüche 1 bis 4 beansprucht und mindestens ein Enyzm.

8. Verfahren zum Herstellen einer flüssigen stabilisierten enzymatischen Zusammensetzung, umfassend den Schritt des Kombinierens von mindestens einem Enzym und einer Formulierung, wie in mindestens einem der Ansprüche 1 bis 4 beansprucht.

9. Verfahren nach Anspruch 8, weiterhin umfassend die Schritte des Mischens des C_2-C_6 mehrwertigen Alkohols und des Wassers und Zusetzen des Poly(cellulose)ethers zu der C_2-C_6 mehrwertigen Alkohol- und Wassermischung vor dem Kombinierungsschritt.

10. Verfahren nach Anspruch 8 oder 9, wobei der C_2-C_6 mehrwertige Alkohol Glycerol, Sorbitol, Propylenglykol, Butylenglykol, Hexylenglykol oder Ethylenglykol ist; wobei die Mischung 1-95 Gew.-% des C_2-C_6 mehrwertigen Alkohols und des Restwassers ist und das mindestens eine Enzym ausgewählt ist aus einer Protease, Amylase, Lipase, Cellulase, Mannase und Xylase.

11. Verwendung einer Formulierung gemäß mindestens einem der Ansprüche 1 bis 4, um eine flüssige enzymatische Zusammensetzung zu stabilisieren, umfassend den Schritt des Kombinierens einer flüssigen enzymatischen Zusammensetzung, welche mindestens ein Enzym enthält, mit der Formulierung.

12. Verwendung gemäß Anspruch 13, wobei der Poly(cellulose)ether ausgewählt ist aus Poly(carboxymethylcellulose) ether und der C_2-C_6 mehrwertige Alkohol Glycerol ist und das mindestens eine Enzym ausgewählt ist aus einer Protease, Amylase, Lipase, Cellulase, Mannase und Xylase.

Revendications

1. Composition de stabilisation d'une composition enzymatique liquide, qui comprend :

   (a) au moins un éther poly(cellulosique),
   (b) un alcool polyhydroxylé en C_2 à C_6, et
   (c) de l'eau,

   où les composants (a) et (b) sont présents en une quantité efficace pour stabiliser au moins une enzyme contenue dans une composition enzymatique liquide.

2. Composition suivant la revendication 1, caractérisée en ce que l'éther poly(cellulosique) est choisi parmi les éthers qui suivent :

   éther poly(carboxyméthylcellulosique),
   éther poly(hydroxypropylméthylcellulosique),

éther poly(hydroxyéthylméthylcellulosique),
éther poly(hydroxybutylméthylcellulosique),
éther poly(hydroxypropylcellulosique), et
éther poly(éthylhydroxyéthylcellulosique).

3. Composition suivant la revendication 1, caractérisée en ce que ledit alcool polyhydroxylé en C_2 à C_6 est le glycérol, le sorbitol, le propylèneglycol, le butylèneglycol, l'hexylèneglycol, ou l'éthylèneglycol et lesdits composants (b) et (c) sont contenus dans un mélange de 1 à 95% en poids dudit alcool polyhydroxylé en C_2 à C_6 et le reste formé d'eau.

4. Composition suivant l'une quelconque des revendications 1 à 3, caractérisée en ce que les composants (a) et (b) sont présents en une proportion combinée efficace du point de vue synergique pour stabiliser au moins une enzyme contenue dans une composition enzymatique liquide.

5. Composition enzymatique liquide stabilisée comprenant une composition suivant l'une quelconque des revendications 1 à 4 et au moins une enzyme.

6. Composition suivant la revendication 5, caractérisée en ce que la composition est une émulsion.

7. Composition enzymatique liquide qui comprend une composition suivant l'une quelconque des revendications 1 à 4 et au moins une enzyme.

8. Procédé de préparation d'une composition enzymatique stabilisée liquide, qui comprend l'étape de combinaison d'au moins une enzyme et d'une composition suivant l'une quelconque des revendications 1 à 4.

9. Procédé suivant la revendication8, comprenant, en outre, avant l'étape de combinaison, les étapes consistant à mélanger ledit alcool polyhydroxylé en C_2 à C_6 et ladite eau, et d'ajouter ledit éther poly(cellulosique) audit mélange d'alcool polyhydroxylé en C_2 à C_6 et d'eau.

10. Procédé suivant la revendication 8 ou 9, caractérisé en ce que ledit alcool polyhydroxylé en C_2 à C_6 est le glycérol, le sorbitol, le propylèneglycol, le buytylèneglycol, l'hexylèneglycol ou l'éthylèneglycol, ledit mélange est constitué de 1 à 95% en poids dudit alcool polyhydroxylé en C_2 à C_6 et du reste formé d'eau, et ladite au moins une enzyme est choisie parmi une protéase, une amylase, une lipase, une cellulase, une mannase et une xylase.

11. Utilisation d'une composition suivant l'une quelconque des revendications 1 à 4, en vue de stabiliser une composition enzymatique liquide, qui comprend l'étape de combinaison d'une composition enzymatique liquide contenant au moins une enzyme et de ladite composition.

12. Utilisation suivant la revendication 13, caractérisée en ce que l'éther poly(cellulosique) est choisi parmi l'éther poly (carboxyméthylcellulosique) et ledit alcool polyhydroxylé en C_2 à C_6 est le glycérol et ladite au moins une enzyme est choisie parmi une protéase, une amylase, une lipase, une cellulase, une mannase et une xylase.